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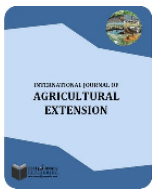

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NEWCASTLE DISEASE AND ITS DIFFERENT APPLICABLE CONTROL OPTIONS IN POULTRY IN ETHIOPIA

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ABSTRACT

Backyard poultry production has been a long-established husbandry practice in Ethiopia. It is estimated that Ethiopia's backyard poultry population is about 53.31 million. The productivity of backyard poultry is constrained by disease outbreak especially Newcastle disease. Newcastle disease is an endemic, highly contiguous, viral disease that affects birds in both intensive and extensive production system. Vaccination against Newcastle disease has been established as one of the many interventions' strategies, geared towards the control of Newcastle disease outbreaks in these flock. Currently, in Ethiopia, four types of Newcastle disease vaccines (HB1, Lasota, IOENDV, and Thermo-stable–12 vaccine) are used for the control of Newcastle disease. The application of conventional vaccination strategy for the control of Newcastle disease has been effectively utilized in intensive poultry production system. However, these conventional vaccination strategies against Newcastle disease outbreaks have not been fully optimized in backyard poultry production. Consequently, the application of thermo-stable vaccine in the form of feed baits seems to be the most appropriate method for effective control of Newcastle disease in village backyard poultry. Many kinds of feed stuff have been tested as a carrier of the vaccine virus; some have been proved unsuitable, while others are relatively suitable. The proper application of vaccine and vaccination programs together with other measures like sanitation, good nutrition, high level of management in most of the commercial poultry farms in Ethiopia and avoiding of concurrent infections, the occurrence of Newcastle disease outbreaks is rarely reported.

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Ethiopia has a huge chicken population with a total population of about 56.1 million which plays a significant role in human nutrition and as an income source (CSA, 2018). Large amount of national egg and poultry meat production are contributed from the traditional poultry production system. However, chicken production is constrained by the disease, predation, lack of management and appropriate breeds in the country (Tadiose *et al.*, 2016); (Terfa *et al.*, 2018)). Among these constraints, disease is the most important and responsible for reducing both numbers and productivity, mortality from egg to adult because of the disease can be estimated to be 80% during some spectacular epidemics (Kinung'hi *et al.*, 2004); Zelalem *et al.* (2014).

Different poultry disease has been reported in Ethiopia, the major cause of economic losses being Newcastle disease (ND), coccidiosis, chronic respiratory disease, Marek's disease, Nutritional deficiencies (Lobago *et al.*, 2005; Mesfin and Bihonegn, 2018). Among the listed disease Newcastle disease is a highly contagious viral

disease affecting wild and domestic avian species (Chaka *et al.*, 2012; Damena *et al.*, 2016). In fact, it has been argued that Newcastle disease may represent the biggest drain on the world economy than any other viral disease (Alexander and Jones, 2003). In Ethiopia the disease known to cause heavy losses in village chicken as compared to commercial poultry farms (Mebrahtu *et al.*, 2018). This is because, unlike commercial chicken, little efforts have been made to control Newcastle disease in Ethiopian village chickens (Desta and Wakeyo, 2012; Bogale *et al.*, 2017).

Of the many intervention strategies to control Newcastle disease, vaccination is the most and highly practical and cost-effective method (Tadiose *et al.*, 2016; Bello *et al.*, 2018). Vaccination provides good means to lessen clinical signs of infection caused by the virulent strain of Newcastle disease virus (Alexander and Jones, 2003; Kapczynski and King, 2005). The conventional vaccine, although they are effective to control Newcastle disease under commercial farms, they require cold chain from their production up to the administration to individual birds (Terfa *et al.*, 2018). Moreover, the relatively large dose of vaccine, the small flock size and scattered presence of chicken, mixing up of multi-aged groups and poor management in the village chickens' system further limit their extensively use in village chickens. This had hindered to design of nationwide preventive strategies against the disease in the village poultry production system. Therefore, the objective of this paper is to review Newcastle disease and its different applicable control options in Ethiopia.

General Account on Newcastle Disease

Newcastle disease (ND) is an acute and highly contagious viral infection that can affect most bird species (Abolnik, 2017). The disease is endemic in many parts of the world and causes high economic losses due to high mortality and reduced poultry production (Qosimah *et al.*, 2018). In rural areas, the disease can kill up to 80% of unprotected poultry and is thereby one of the biggest constraints to village poultry production and a considerable restrict of rural development (Alexander *et al.*, 2004; Tadiose *et al.*, 2016; OIE, 2013).

Etiology

Newcastle disease is caused by avian Paramyxovirus serotype -1 (APMV -1) (Radositis *et al.*, 1997). This serotype together with other eight APMV serotypes (APMV-2 to 9) has been recently placed in genus Avulavirus, family paramyxo viridae (OIE, 2013). A new serotype of APMV (APMV-10), isolate from penguins has been confirmed (Miller *et al.*, 2010). This family of viruses contains a single-stranded RNA in the helical symmetry of nucleocapsid and has an outer envelope (Kapczynski *et al.*, 2013; Cao *et al.*, 2013). The Newcastle disease virus encodes by six major proteins that comprise the nucleoprotein (NP), phosphoprotein, Matrix, Fusion, Hemagglutinin Neuraminidase (HN) and RNA dependent RNA polymerase protein (Cornax *et al.*, 2013). The matrix protein forms a linkage between the glycoprotein in the virus envelop and nucleoprotein in the nucleocapsid thus stabilizing the virus structure. The F gene and the HN gene encode essential proteins for virulence determination. The fusion (F) protein is responsible for mediating fusion of the viral envelope with cellular membranes while the HN protein is involved in cell attachment and release (Heiden *et al.*, 2014; Qiu *et al.*, 2014). The HN and F proteins are the main targets for immune response to NDV (Chaturvedi *et al.*, 2011; Kumar *et al.*, 2011). Moreover, HN and F glycoproteins are essential for virus-neutralizing antibodies (Solemen, 2003).

Originally strain of Newcastle disease virus was differentiated according to the mean death time (MDT) of the chicken embryo after infection, by determining the intracerebral pathogenicity index (ICPI) on day-old chick (Doc) after intracerebral infection and by determining intravenous pathogenicity index (IVPI) on 6 weeks old chicken after intravenous (Nakamura *et al.*, 2014). Strains of Newcastle disease virus differ widely based on their virulence, tropism to organs and as well as their capacity to spread, in which case some are highly pathogenic, some are moderately pathogenic, while but some others are mildly pathogenic (Alexanderand and Senne, 2008).

Velogenic strains are markedly pathogenic and cause an acute infection of all ages of chickens. It is characterized by lesions in the respiratory tract, visceral organs, and brain. Its infections spread rapidly amongst susceptible flock and with up to 90% morality (Shankar, 2008). This strain can be classified as velogenic viscerotropic Newcastle disease (VVND) and velogenic neurotropic Newcastle disease (VNND) based on the site where the virus causes lesions (Alexanderand and Senne, 2008). Mesogenic strains are moderately pathogenic and cause an acute respiratory and sometimes lethal nervous

system infection of young chickens, but in older chickens' mortality is rare, even though it affects egg quality and production (Abdisa and Tagesu, 2017). Pathogenic strains are mildly pathogenic and cause a mild or in apparent respiratory tract infection of chickens with negligible mortality. Several pathogenic strains are widely used as a vaccine (Kahn *et al.*, 2005).

Table 1. Examples of pathogenicity indices of strains of Newcastle disease virus.

Virus strains	Pathotype	MDT	ICPI	IVPI
HBI	Lentogenic	120	0.2	0
Lasota	Lentogenic	103	0.1	0
Muket Swar	Mesogenic	96	1.4	0
Roakin	Mesogenic	68	1.45	0
GB-Texas	Velogenic	55	1.75	2.7
Milan	Velogenic	50	1.9	2.8

MDT = Mean Death Time (in hour); ICPI = Intra Cerebral Pathogenicity index; IVPI = Intravenous Pathogenicity index.
Source: (Nasser *et al.*, 2000).

Sources and transmission of Newcastle disease virus: The sources could be any excretion like fine aerosols, dropping from nasal cavity and mouth and faces from clinically diseased or carrier chickens (Alders and Spradbrow, 2001; Mesfin and Bihonegn, 2018). Contaminated feed, water, and equipment may serve as a source of infections, an egg laid by infected hens; live vaccines also contribute as the reservoir of Newcastle disease virus. The importation of birds that have access to enter into the flock may also serve as a source of infection (Brown and Bevins, 2017).

Exposure of susceptible chicken to any of these sources of viruses can result in the transmission of the virus. If the egg which contains the virus is hatched and accidentally broken in the hatchery, the entire hatches of chickens may be exposed and infected (Getabalew *et al.*, 2019). The aerosol is the main mode of transmission in an intensive poultry production system; this is because of confinement, whereas transmission by the oral route is the main mode of transmission of the virus in free-ranging scavenging village chickens (Caupa and Alexander, 2009).

Control and Prevention

Sanitary and management measures: These are the ways that enable to reduce or limit the contact of susceptible chickens with the virus by doing so; it limits the spreading of disease (Markos and Abdela, 2016). The hatchery must be isolated from processing plants or operations. It is particularly important to avoid the use of egg form flock showing a significant drop in egg production (Udo *et al.*, 2006). Sick chicken should be isolated from the flock if there is an outbreak in the flock; the affected flock should be quarantined and destroyed. The dead chickens should be a burn and buried to avoid contact of the virus with the healthy chickens. Avoid any other possible sources of infection like manure, or litter should be disposed at proper site and burning should be applied, limit movement of personnel, proper changing of clothes and footwear during the visit, cleaning and washing of each affected house and equipment particularly after and outbreak. Adequate ventilation is essential in the flock; which is kept in an intensive production system (CFSPH, 2016). Restriction of movement of poultry and poultry products from one flock to another flock, the water and feeds should be clean. Avoid reservoir of the virus from flock like convalescence village chicken, which harbors the agents, other species of poultry like guinea fowl, pheasant, geese, peacock (Yune and Abdela, 2017). Wild birds contain velogenic, mesogenic and lentogenic strain of the virus and these are considered as reservoirs and sources of dissemination of virus to susceptible village chicken (Bello *et al.*, 2018). Animals like dogs, cats, fox, and rodents shed the virus in their feces for as long as 72 hours after having eaten fouls carcasses so that they cause infection in the flock if they have access to contact with the chicken. So, it should limit the contact of these animals with chickens (Delahoy *et al.*, 2018).

Vaccination

Vaccination against Newcastle disease can be performed

using either live or killed vaccine (Shim *et al.*, 2011). Although this strategy has reduced the disease rate after vaccination, it may also have contributed to the fact that the current vaccine and vaccination campaigns are not maximally effective in preventing infection and transmission (Senne *et al.*, 2004; Mesfin and Bihonegn, 2018). The effectiveness of the Newcastle disease vaccine in control of the disease depending on the virulence of field strain, the immunological state of birds, disease status of birds to be vaccinated, the method of vaccination and the types of vaccine used (Nasser *et al.*, 2000). The subject of vaccination against Newcastle disease is complex and it is recommended that when evaluating the efficacy of vaccine under field condition, the immune response should be monitored to establish the level of immunity under specific conditions. Not all birds respond to vaccination in the same manner (Senne *et al.*, 2004). The variation may be due to faulty administration of vaccination and impaired by various infection diseases like infectious bursal disease (IBD) (Bello *et al.*, 2018).

Live Vaccines

A live vaccine of Newcastle disease has been used by the poultry industry for many years. Vaccination of large numbers of chickens against ND is usually carried out using a non-virulent live virus that is administered by spray or drinking water (van Boven *et al.*, 2008). This administration technique usually produces considerable variation in individual antibody immune response of vaccinated birds, indicating potential variation in the level of protection after vaccination (Senne *et al.*, 2004). The strain used for the preparation of live vaccine can be broadly categorized as lentogenic and mesogenic strains (Nasser *et al.*, 2000).

Lentogenic Strains Live Vaccine: most live vaccine currently used in most countries is derived from this strain. The best-known stains of lentogenic strain live vaccine are HB1, F1 and lasota. In recent years other lentogenic strains have been examined for used safely and effectively in all chicken classes. The lasota strains are more invasive and immunogenic than HB1 strain and have a good boosting immunity (Nasser *et al.*, 2000). The HB1 and F1 vaccine doesn't cause nervous disease in day-old chicks unless given intra cerebrally but may cause mild and transitory respiratory symptoms. The F1 strains cause the least reaction, the HB1 strain generally has little or no clinical effect whereas the lasota strains cause more post vaccine symptoms (Abel, 2018). Cloning was used to obtain viruses with high immunogenicity combined with acceptable vaccination reactions. Furthermore, an in ovo injectable vaccine was tested and found to be effective (Dimitrov *et al.*, 2017). This route of vaccination could kill or weaken the embryo unless the virus is treated with alkylating agents, ethyl methane sultanate, to cripple the virus (Solemen, 2003). Effective vaccination requires ideally that all birds in a flock get vaccinated, since the spread of lentogenic viruses may be limited by individual application, an eye, and nose drop is preferred to obtain a uniformly high level of protection. However, the individual application is too laborious and there is practiced in small flock size only, as a result, mass application using spray or aerosol equipment or via the drinking water is favored because it is cheap and convenient. Moreover, this application method triggers local cellular and humeral immunity in the respiratory tract preventing infection of mucosal surfaces or reducing virus replication at this site. As a result, virus invasion to systemic tissue is blocked. Different in effect of spray and aerosols are caused by the size of droplets, coarse droplets are short-lived whereas fine droplets are long-lived. Vaccination through water can also be used, but give varying results due to the variation in water intake between and among birds (Mebrahtu *et al.*, 2018).

Mesogenic Strain Live Vaccine

the most widely used vaccine included in this category is roakin, komarou, hert ford shire (Herts), MK 107 and muketswar. The roakin, komarou, herts and muketswar strains are widely used throughout Africa, the Middle East and south East Asia (Czegledi *et al.*, 2003). These vaccines are administered by parentral routes and are not recommended for immunization of chicken under eight weeks of age nor for young pullets or adults not previously immunized with lentogenic strain. They could produce serious problem in fully susceptible poultry (Zhao *et al.*, 2012).

The immunity elicited by live vaccine administration should appear within five to seven days after vaccination and be of substantial degree after the second week. The duration of immunity from live vaccine may be varying greatly from flock to flock and among individuals. It may wane appreciable within two months and revaccination is recommended within two months to a year (Bello *et al.*, 2018). Effective immunity is achieved after two or more

exposure to a vaccine (Alders and Spradbrow, 2001).

Inactivated Vaccine

Inactivated ND vaccine is prepared by growing antigenic strain of a virus on embryonated egg harvesting the dead or dying embryos and tissue and inactivating the virus, usually by chemical agents such as formaldehyde, crystal violet or beta propriolacton (Chowdhury *et al.*, 2015). Specific immunity against ND develops after two weeks in ten days of age or older healthy birds when vaccinated and the immunity may decline considerably in two six months after vaccination. Therefore, a minimal period of nine weeks is required between initial vaccination and revaccination (Alders and Spradbrow, 2001). This vaccine is administered through parental routes; gives a high level of protective antibodies that persist for a long period of time. Since inactivated vaccines are more laborious and time-consuming to produce and require individual applications, their use is extremely expensive (Solemen, 2003).

Recombinant Vaccine

The major drawback of all currently used whole-virus-based live and inactivated NDV vaccines is that vaccinated animals cannot be distinguished from infected animals with standard serological tests, such as hemagglutination inhibition (HI) or virus neutralization (VN) tests (Milic *et al.*, 2017). Newcastle disease virus has two surface glycoproteins, fusion (F) and haemagglutinin/ neuraminidase (HN) (Getabalew *et al.*, 2019). The gene coding for either of these can be inserted into a different kind of virus to make a recombinant vaccine. For instance, the fusion gene inserted in herpes virus of turkeys produced a vaccine which gave good protection against virulent NDV (Sadigh *et al.*, 2018; Smietanka *et al.*, 2019). One advantage of this method is that the host virus may have better stability than NDV. Another advantage is that antigen for multiple different pathogens can be inserted into the same host virus to produce a single vaccine against several different diseases. Perhaps the most significant advantage for field use is that it is possible to the response to the vaccine independently of the wild virus but in its presence and conversely it is possible to detected antibodies against the wild virus in the presence of vaccination (Alexander *et al.*, 2004). This is done by using an enzyme-linked immunoabsorbent assay (ELISE) that uses a purified antigen and comparing the results with those of an ELISA using a whole virus antigen. An ELISA was prepared using only nucleocapsid protein of NDV as antigen. This detected antibodies against wild virus, but not antibodies against a recombinant fowl pox virus expressing HN glycoprotein. A parallel ELISA using whole virus as antigen detected antibodies against the vaccine (Bello *et al.*, 2018). A disadvantage of recombinant vaccine is that where they have been developed commercially the cost is high (Alexander *et al.*, 2004; Getabalew *et al.*, 2019). Novel recombinant baculovirus vaccines expressing the NDV F or FN genes were designed. The F-series of vaccines provided a greater degree of protection (87.5-100%) than the HN series (62.5-87.5%). The baculovirus system is a promising plant form for NDV vaccine development that combines the immune stimulatory benefits of a recombinant virus vector with the non-replicating benefits of a DNA vaccine (Ge *et al.*, 2016).

Newcastle Disease in Ethiopia

Newcastle disease (ND) is the most important cause of economic losses to poultry production in Ethiopia. The disease has different local names, but the most commonly used is “Yedoro Fengle” (Chaka *et al.*, 2012). The ND virus involved was velogenic strain and cause some 80% mortality. Recent studies in Ethiopia have confirmed the presence of both velogenic and lentogenic strains circulating within rural household flocks (Chaka *et al.*, 2013; Fentie *et al.*, 2014).

Prevalence of Newcastle Disease in Ethiopia

In Ethiopia, different studies were carried out various times to identify constraints of village poultry production under a traditional management system. The result indicates that disease is the most limiting factor followed by predation, external parasites and feed shortage (Moges *et al.*, 2010; Birhan, 2014). The studies done in three states farms revealed that nine outbreaks had occurred and caused 14.9% mortality in vaccinated flocks. The sources of the outbreak were difficult to trace, but management and hygienic conditions, nutrition, type of vaccine used and concurrent disease all might have contributed to the introduction, spread, and persistence of the disease (Mesfin and Bihonegn, 2018). Multi–aged groups in the farms, minimal distance and lack of separation between different units, poor disposal of dead birds, absence of all-in- all-out system and maintaining different types of birds in the same farms created favorable conditions for the outbreak and for the

persistence of the disease in the farm. Well-fed chickens can respond satisfactorily to vaccination and attain sufficient protective antibodies. Nutritionally deficient chickens are more susceptible to disease as a result of poor antibody response after vaccination (Nasser *et al.*, 2000).

The epidemiology of Newcastle disease in village poultry in Ethiopia is poorly understood and there is no appropriate control strategies designed against the disease. This is partly due to a lack of disease monitoring capacity in country (Terfa *et al.*, 2018). Consequently, farmers start to consider losses due to the disease as normal and natural, and they fail to report an outbreak to the veterinary authorities (Dessie and Jobre, 2004). In Ethiopia Newcastle disease occurs almost any time of the years, inflicting heavy losses and velogenic strains that are widely distributed throughout the country. Newcastle disease becomes a problem throughout the year after villagization program in 1984. However, the existence of few clinical cases of Newcastle disease apart from the higher seroprevalence of antibodies against Newcastle disease virus during the dry period of the year, also suggests that the disease may probably be more serious in the rainy seasons (Sahlu *et al.*, 2015). Belayheh *et al.* (2014) has examined 355 non-vaccinated village chickens in Kersana-Kondalaity district in Ethiopia and 5.6% seroprevalence was recorded. Jarso (2016) reported that the seroprevalence of 28.6% were found in East Shewa zone Ethiopia.

A Newcastle disease (ND) seroprevalence of 27.9% in Agarfa and Sinana districts Ethiopia was reported among local scavenging chickens kept under a traditional management system (Geresu *et al.*, 2016). Another study was conducted in two districts of eastern Shewa zone, Ethiopia by Chaka *et al.* (2012) to estimate the seroprevalence of Newcastle disease in the wet and dry seasons.

The overall seroprevalence of Newcastle disease was 5.9 % during the dry season and 6.0 % during the wet season.

Control of Newcastle Disease in Ethiopia

Effective control of Newcastle disease can be achieved by maintaining good hygiene, avoiding concurrent infection, maintaining good nutrition, quarantine, high level of management and appropriate vaccination, and also depopulation, in combination with monitoring of the antibody after vaccination (Nasser *et al.*, 2000; Bello *et al.*, 2018). These measures could be grouped into sanitation and management measures, and vaccination strategies.

Sanitation and Management Measures

These are the ways that enable to reduce or limit the contact of susceptible chicken with the virus by doing so it limits the spread of the disease (Abdi *et al.*, 2016). The implementation of these measures largely depends on the degree of awareness of the personnel involved and facilities available. Moreover, it is highly variable from farm to farm and among the different production systems. For instance, the sanitary and management control measures were not efficient and have contributed significantly to the occurrence and severity of series of outbreaks of ND from 1983-1995 in three sate poultry forms in Ethiopia (Yune and Abdela, 2017; Mebrahtu *et al.*, 2018).

Vaccination Program

Among the different intervention strategies to control Newcastle disease, vaccination is the most and highly practical and cost-effective method that ensures successful poultry production via maintaining poultry health at a high level (Al-Garib *et al.*, 2019; Mesfin and Bihonegn, 2018). Currently, in Ethiopia, four types of ND vaccine (HB1, Lasota, thermostable and IOE ND vaccine) have been produced. Although the conventional vaccines locally produced are effective to control Newcastle disease under commercial farms, they require cold chain from their production up to the administration to individual birds and handing of each bird, these, therefore limit their extensively use in village chicken (Abdi *et al.*, 2016; Terfa *et al.*, 2018). It should be also noted conventional vaccine application to individual birds mainly via the ocular or oral route, whereas the inactive type of vaccine is given through injection to individual birds, sub-cut is at the back of the neck or intramuscularly in the breast muscle (Mustafa and Ali, 2005).

The Prospect Use of the Thermo-Stable Vaccine

The recently developed heat resistant V4 vaccine does need a cold chain, in contrast to the conventional vaccine (HB1 and Lasota). It can be delivered by the oral route and can be applied by unskilled personnel under field conditions. Several studies confirmed that the V4 vaccine is highly protective when given by conventional routes (Mebrahtu *et al.*, 2018). The thermo-stable–12 vaccine produced by NVI and was kindly granted by pan African veterinary vaccine center (PAVVC). This vaccine

is the same as the thermo-stable V4 vaccine. The thermo-stable vaccine can be applied through ocular routes (Nega *et al.*, 2012). Proper use of vaccine and vaccination program together with other measures like sanitation, good nutrient and high level of management, and avoiding of concurrent infections, the occurrence of ND outbreak become rare. However, the disease is still the main constraints throughout the years in village chicken (Tulu, 2019). The problem with controlling ND in village chickens are multiple age group of flock scattered over extremely large areas, lack of physical separation over the unconfined flock, frequent introduction of young susceptible chicken to the group, poor nutrition, poor marketing and transporting facilities and lack of education to farmers (Nasser *et al.*, 2000). On the other hand, lack of cold chain and difficultly in catching these birds result in difficultly in use of conventional vaccination program in village chicken against Newcastle disease (Abdi *et al.*, 2016). However, the development of thermo-stable-12 vaccines and its application through feed based show a bright future to control ND in village chickens (Terfa *et al.*, 2018). The advantage of these vaccines includes: its easy application through feed carrier can be applied by unskilled personnel, low cost, don't need a cold chain and lateral spread of the vaccine virus within vaccinated and contact flock. These features make the new vaccine particularly suitable for application in Africa as it has been proved to be effective in trials done in East African countries (Alders *et al.*, 2019).

Feed Trials for Carriers of Newcastle Disease Virus–12 Vaccine

Oral vaccination with thermo-stable strains of NDV is the only feasible way of reaching large, scattered populations of free-ranging, scavenging, nearly feral chickens (Echeonwu *et al.*, 2007; Tadiose *et al.*, 2016). The efficacy of the thermo-stable vaccine was tested in many countries under laboratory and field conditions on different grains as a carrier and, the result with feed as a vaccine was variable, some grains were found to be suitable vaccine carriers, others were not. This was as a result of failing to take up the virus, inactivating the virus or failing to release the virus (Nasser *et al.*, 2000; Abdi *et al.*, 2016).

The ideal food carrier for the avian viral vaccine would be cheap, harmless to the virus and acceptable to the chickens. The role of the carriers would be simply to absorb the virus from an aqueous suspension and to release it in viable form in the digestive tract of a chicken. Food vaccines have been tested by feeding them to chicken and measuring either the antibody response or resistance to challenge (Lawal, 2016; Abdi *et al.*, 2016).

In Ethiopia, different authors undertook few trials by considering the situation of ND in village chickens, advantage of thermo-stable –12 vaccines and positive experience of some countries in the control of ND in the village chicken by using the V4 vaccine. Recent study conducted in Ethiopia on five cereal grain species in different forms were evaluated for suitability and efficacy as a carrier for the ND12 vaccine. The result indicated that cracked maize, parboiled barley, untreated and parboiled wheat in addition to parboiled sorghum would be promising suitable carriers for large scale administration of thermo-stable –12 vaccines under Ethiopian field (village) conditions (Abdi *et al.*, 2016). A previous study also carried out in Ethiopia shown that Newcastle disease virus thermo-stable–12 vaccine mixed with barely and fed immediately, resulted in sero-conversion and protected 100% of the vaccinated chicken after the third vaccination, and also intraocular group were found protected 100% after the third vaccination whereas the wheat and maize group give raise to unsatisfactory results (Nasser *et al.*, 2000). In study conducted using bran, ground grain and water as a vehicle by Abdu *et al.* (2012) and Mebrahtu *et al.* (2018) water vaccination was more protective than vaccination using feed as a channel. The difference in the immune response of chicken on vaccinated with water and feed is the time taken to formulate vaccine. It is taking longer in feed channel than that of water. This is mainly related with inadaptability of the chicken for the feed that the vaccine is constituted. It is believed that prior adaptation for the grain in which that vaccine was given may be increase the efficiency of the vaccine. Other study conducted by Musa *et al.* (2010) the mortality of chicken that were vaccinated with vaccine treated sorghum is devastating (up to 100% mortality). The finding of this study on treated barley is different from the findings of Nasser *et al.* (2000) which report more than 90% protection. This might be due to the number of animals under the challenge and the difference in the type of chicken used in the treatment Tadiose *et al.* (2016). The intraocular group protected 100% earlier as compared to the rest group of routes. But this route

needs complete immobilization of chicken, which is not practical in rural areas of the country where village chickens are semi-domesticated or wild. Therefore, they conclude that the barely based Newcastle disease thermostable–12 vaccine could be used to vaccinate village chickens. Nasser *et al.* (2000) showed that uncooked barely and sorghum as vaccine carrier was not entirely satisfactory, and the chickens neither seroconverted nor were protected against the challenge with virulent NDV, unless the grain feed immediately after mixed. However, boiling, washing and dying of the grain before mixed with vaccine give satisfactory protection as late as 14 hours after mixing (Nasser *et al.*, 2000; Abdi *et al.*, 2016).

Survival of Vaccine Virus on Food Carriers

Two basic methods are available to investigate the survival of vaccine virus on food stuffs; the first involves coating virus on to the feed particles and observing for recovery of the virus from feed particles. Low levels of recovery are difficult to interpret such results may indicate the inactivation of the virus, but they may also represent irreversible binding or even an initial failure of the attachment. The second procedure involves feeding the treated grain to chicken and examined for either antibody response or resistance to infection (Abdi *et al.*, 2016; Tadiose *et al.*, 2016).

Recovery of virus from uncooked grain was usually poor, especially after storage for even a few hours and antibodies response in chicken after feeding also poor. This is mainly associated with grain factor. In contrast, par-boiled grains were significantly extended the survival of the virus, and recovery of the virus from the grain was possible as late as 14 hours when kept at room temperature and recovery of the virus from non-boiled grains was possible only immediately after mixing (Nasser *et al.*, 2000; Mebrahtu *et al.*, 2018). In elsewhere, a proportion of the vaccine carried to the village for feeding to chicken has been returned to the laboratory for titration, and then recovery of virus from cooked white rice was high, while recovery from uncooked rice was lower and variable (Echeonwu *et al.*, 2007). Significantly extend survival of the virus on treated grain, might be this treatment leads to better attachment or absorption of the virus to interior ports of the grain, rather than to the outer side of the grain where the antimicrobial are concentrated and also the treatment destroys any antimicrobial substances of the grain (Nasser *et al.*, 2000).

The various additives have been used to improve the survival of the ND vaccine, polyvinyl pyrrolidone (PVP) has been very successful in elsewhere. Polyvinyl pyrrolidone (PVP) is used to counter the toxicity of legume seed. Sucrose seems to be a useful additive and counter bacterial growth, although it will permit fungal growth. Skim milk powder is useful for short-term protection but spoils very rapidly at room temperatures (Echeonwu *et al.*, 2007). Methylcellulose has been used in elsewhere. It gives similar protection to PVP but is more difficult to use, it could warrant further investigation of grains where viral adhesion seems to be a problem. Gelatin has been used with vaccines other than ND vaccines but it would not be an acceptable additive in most countries (Olabode *et al.*, 2010).

Innate immune response to Newcastle disease infection virus in poultry: The innate immune response comprises factors exist prior to the advent of infection and are capable of exclusion or rapid response to microbes. The primary components of innate immunity of poultry are (1) physical and chemical barriers, such as feathers and skin, epithelia and production of mucus; (2) phagocytic cells, including macrophages and natural killer cell; (3) complement proteins and mediators of inflammation and (4) cytokines (Miller *et al.*, 2010). The innate immune response to virus infection is immediate reactions intended to control and inhibit virus growth and spread and aid in developing pathogen-specific protection through the adaptive immune response. The early reaction of the innate system uses germ-line encoded receptors, known as pattern recognition receptors (PRR's), which recognize evolutionarily conserved molecular marker of infectious microbes, known as PAMP's (pathogen-associated molecular patterns). Recognition of PAMPs by PRRs, either alone or in heterodimerization with other PRRs, (toll-like receptors (TLR)) nucleotide-binding oligomerization domain proteins (NOD); RNA helicases, such as retinoic acid-inducible gene 1 (RIG-I) or MDA5; C-type lectins), induces intracellular signals responsible for the activation of genes that encode for pro-inflammatory cytokines, anti-apoptotic factors, and antimicrobial peptides (Kapczynski *et al.*, 2013). The virus is first recognized by host sentinel proteins, including TLR and NOD proteins, producing rapid signaling and transcription factor activation that lead to production of soluble factors, including interferon and cytokines,

designed to limit and contain viral replication. The NDV infection in vitro results nitric oxide (NO) induction in chicken heterophils and peripheral blood mononuclear cells, interferon alpha (IFN-a) and beta (IFN-b) mRNA detection in macrophage and gamma (IFN-c) mRNA production in peripheral blood mononuclear cell (Ahmed *et al.*, 2007; Sick *et al.*, 2000). Moreover, infection of chicken heterophil decreased the ability to phagocytose bacteria, resulting in impaired heterophil function and making birds more susceptible to secondary infection. Constitutive low-level expression of NO in the vascular endothelium plays a beneficial role in maintaining blood vessel homeostasis, but high level of NO produced by macrophage in response to pathogens can have toxic effects on the host (Kapczynski *et al.*, 2013).

Adjuvant to improve the immune response of NDV vaccines were initially focused on inactivated vaccine, however, now includes substance to favorably modulate the immune response from live NDV vaccines. Dietary supplements are commonly tested because the compounds may be locally available and/or because the compound maybe easily added to the diet to improve the immunity after vaccination. Lactobacillus-based probiotics have been shown to improve humoral immunity to live NDV vaccines in birds under heat stress (Sohail *et al.*, 2010). Antibiotics may be added to water at the time of vaccination to provide an undefined benefit to the birds (Khalifeh *et al.*, 2009). However, when antibiotics are evaluated for their ability to positively potentiate the humoral immune response to NDV vaccines, typically they are found to decrease the response or not significantly improve the response (Munir *et al.*, 2007). Astragalus polysaccharides commonly used in Chinese medicines to enhance the immune response demonstrated slight improvements in the humoral immune response to NDV vaccination with or without sulfation (Huang *et al.*, 2008). Glycyrrhetinic acid liposomes demonstrated a significantly improved humoral response to NDV vaccination 21–42 days after vaccination (Zhao *et al.*, 2011).

Cellular Immunity Induced by Newcastle Disease Virus

Cell-mediated immunity (CMI) is specific adaptive immunity mediated by T lymphocytes and has been suggested to be an important factor to the development of protection in chickens vaccinated against NDV and contribute to viral clearance. Cell-mediated stimulation following NDV infection is detected as early as 2-3 days post infection. Studies also confirmed that CMI responses to NDV may be detected shortly after vaccination with a live NDV vaccine. Result of these shown that antibodies are the key modulators of protection, but that CMI likely contribute to decrease viral shedding through target killing of NDV infected cells (Lwelamira *et al.*, 2009).

Other studies have compared CMI responses between birds receiving live versus inactivated NDV vaccine. In one study, measurement of IFN-c by ELISA and proliferation to NDV from splenocytes obtained from chickens receiving live or inactivated NDV vaccines were compared. Results indicate increased CMI with the live NDV vaccination. Whereas, live NDV stimulates both major histocompatibility complex (MHC) class I (CD8+) and II (CD4+) presentation in the host, CMI derived from inactivated NDV vaccines take longer to develop and are not as robust. Additional, studies examined the role of vaccine virulence in CMI. Not surprisingly, the virulence of the virus appears to play a role in CMI stimulation. Rauw *et al.* (2009) demonstrated an earlier and shorter CMI induced by a less virulent NDV vaccine strain, compared to a stronger and longer CMI mediated by a more virulent vaccines strain. Thus, the more virulent strain persisted longer in the bird and therefore was able to increase magnitude and duration of CMI.

In the chicken, IgM, IgY (avian IgG equivalent) and IgA antibodies are produced as part of the immune response. Antibodies are detected at the site of infection and in the blood starting at six days after infection or live virus vaccination and peaks 21–28 days after infection. Antibodies neutralize the ND virus particles by binding and preventing attachment of the virus to host cells (Al-Garib *et al.*, 2019). Approximately 30% of the IgY and 1% of the IgM and IgA antibodies present in the hen plasma will passively transfer to the offspring and if the NDV antibody levels are high enough can provide protection until the levels fall below a protective level. This maternal antibody can interfere with live vaccination by neutralizing the vaccine virus (Hamal *et al.*, 2006).

CONCLUSION AND RECOMMENDATIONS

In Ethiopia, Newcastle disease is an endemic poultry disease and known to cause heavy losses in both commercial and village chicken, but the degree of loss is

higher in village chickens. Effective control of Newcastle disease is very complex because it requires consideration of different factors. Of these control strategies, vaccination is the most and highly practical and cost-effective method. The conventional vaccines although they are effective to control Newcastle disease under commercial farms, they have not been utilized in the village chicken production system, due to different reasons. Consequently, thermo-stable vaccine applied as feed baits seem the most appropriate method for effective control Newcastle disease in village chickens. With proper use of vaccine and vaccination programs together with other measures like sanitation, good nutrient, high level of management in most of the commercial poultry farms in Ethiopia and avoiding of concurrent infections, the occurrence of Newcastle disease outbreak become rare. Based on the above conclusion, the following recommendations are forwarded.

- The epidemiology of Newcastle disease in village chicken should be studied in detail in order to design appropriate control measures.
- In village chicken, in addition to sanitation and other measures the use of feed-based thermo-stable –12 strain vaccine should be encouraged through extension services.
- Further studies should be conducted on different types of feedstuff as a carrier of thermo-stable –12 strain vaccine.
- Distribution of exotic breed of chicken which has high production potential and adaptability to the tropical environment should go together with feeding, housing and health care packages through extension services to the rural farmers.

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